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TITLE: Study the Origin and Mechanisms of Castration Resistance Characterized by Outgrowth of Prostate Cancer Cells with Low/Negative Androgen Receptor

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14. ABSTRACT

While the association of androgen receptor (AR) amplification with poor clinical outcome is well known in prostate cancer, the impact of AR signaling level to treatment response has not been studied. Our data suggests that in primary prostate cancer where there are rare events of AR amplification, the level of AR activity might be able to distinguish aggressive disease. When we isolated cells with low and high AR activities, the tumors derived from cells with high AR activity acquired resistance to enzalutamide faster, implying the clinical implication of varying AR activities. We also identified three AR regulated genes, GREB1, KLF8 and GHRHR, upregulated in cells with high AR activity and promoted AR transcriptional output in a feedback mechanism. Given the known function of GREB1 as an ER cofactor, we selected GREB1 for further study and found that GREB1 amplifies AR transcriptional activity and potentially promote enzalutamide resistance. Further understanding of the molecular function of GREB1 will provide novel insights into the development of effective therapeutic approaches to treat enzalutamide resistant prostate cancer.

15. SUBJECT TERMS
Study the molecular characteristics and enzalutamide sensitivity of prostate cancer with different AR activity.

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Introduction

Given the key role of androgen receptor (AR) signaling in disease progression, the current approach to treat prostate cancer is AR-targeted therapy. While this initially results in tumor regression, aggressive disease typically recurs, making the treatment of what is now called castration-resistant prostate cancer (CRPC) the major challenge in the field (1, 2). In CRPC, conventional AR therapy fails to block AR activity as the cancer cells restore AR signaling, primarily through amplification of AR (3). AR is amplified exclusively in metastatic CRPC, where more than 50% of tumors harbor AR amplification (4). Furthermore, recent study showed that AR amplification was found in circulating tumor cells (CTCs) of patients undergoing treatment of the second-generation AR inhibitors (5), enzalutamide (6) and/or abiraterone (7), highlighting the importance AR amplification as a drug resistant mechanism in prostate cancer.

Most men with prostate cancer have localized disease at the time of the diagnosis, but the clinical response of primary prostate cancer is highly variable, reflecting the heterogeneity of the disease (8). Indeed, several recent studies have classified primary prostate cancer based on the recurrent genomic alterations, highlighting the molecular and genetic diversity across the tumors (9-12). Additional interesting data from this study was that when the AR activity score was analyzed with the expression level of AR target genes (13), wide spectrum of AR score was observed across the primary prostate tumors (10). In fact, previous studies have asked if AR activity can predict disease progression, and showed that AR nuclear level had positive correlation with reduced time to PSA increase after radical prostatectomy (14), and AR signaling output might increase radioresistance by promoting expression of DNA repair genes (15). However, the impact of differential AR activity, not AR level, on the clinical response to the next generation AR targeted therapy has not been studied.

In this study, we asked if prostate cancer cells with different AR activity respond differently to the second-generation AR inhibitor, enzalutamide, and what makes the cells have varying AR activities. Our data showed that the cells with different AR activities have distinct molecular characteristics that result in different sensitivity to enzalutamide treatment. We also identified an AR cofactor, GREB1, drives differential AR output in prostate cancer cells that might have clinical implication.

Keywords

Accomplishments

Specific Aim 1: Determine if prostate cancer cells with low AR activity are less sensitive to enzalutamide

1-1. Determine if the different AR activities within prostate cancer cells are stable phenotype

Proposed completion date: 09.2015-05.2016 (9 months)

Actual completion date: 09.2015-02.2016 (6 months)

Percentage of completion: 100%

Results: To isolate cells with different AR activities, we infected prostate cancer cells with lentivirus containing an eGFP AR reporter construct where the eGFP expression is driven by the probasin promoter modified to contain three AR responsive elements (16). The cells were infected at low multiplicity of infection (MOI) to enable each cell has one copy of reporter construct. Cells stably integrated with the construct were selected by mCherry flow cytometry (Figure 1A). In situ analysis shows that the LNCaP reporter cells express different level of eGFP while having similar level of AR analysed by immunofluorescence staining (Figure 1B), which was confirmed using flow cytometry where we observed wide range of eGFP AR reporter activity (Figure 1C).

To isolate cells with low (AR-activity-low, AR-low) and high (AR-activity-high, AR-hi) AR activity, we sorted out 5% of the entire population with lowest and highest eGFP expression, respectively (Figure 1C). As expected, AR-hi cells have higher endogenous AR target genes expression than AR-low cells (Figure 1D,E), indicating that the reporter construct used in this study accurately reflects the endogenous AR activity. The sorted AR-low and –hi cells maintained their AR activities over 30 days (Figure 1F), demonstrating the stability of their phenotype.

To determine mechanisms underlying the different AR output, we compared AR level between AR-low and –hi cells. Importantly, the two populations have comparable AR expression shown by both mRNA and protein levels (Figure 1D,E), suggesting that there are other factors than AR alone causing different AR output between the two populations. Furthermore, AR-hi cells showed enhanced up-regulation of AR target genes in response to DHT treatment (Figure 1G), suggesting that AR-hi cells have enhanced AR transcriptional activity.
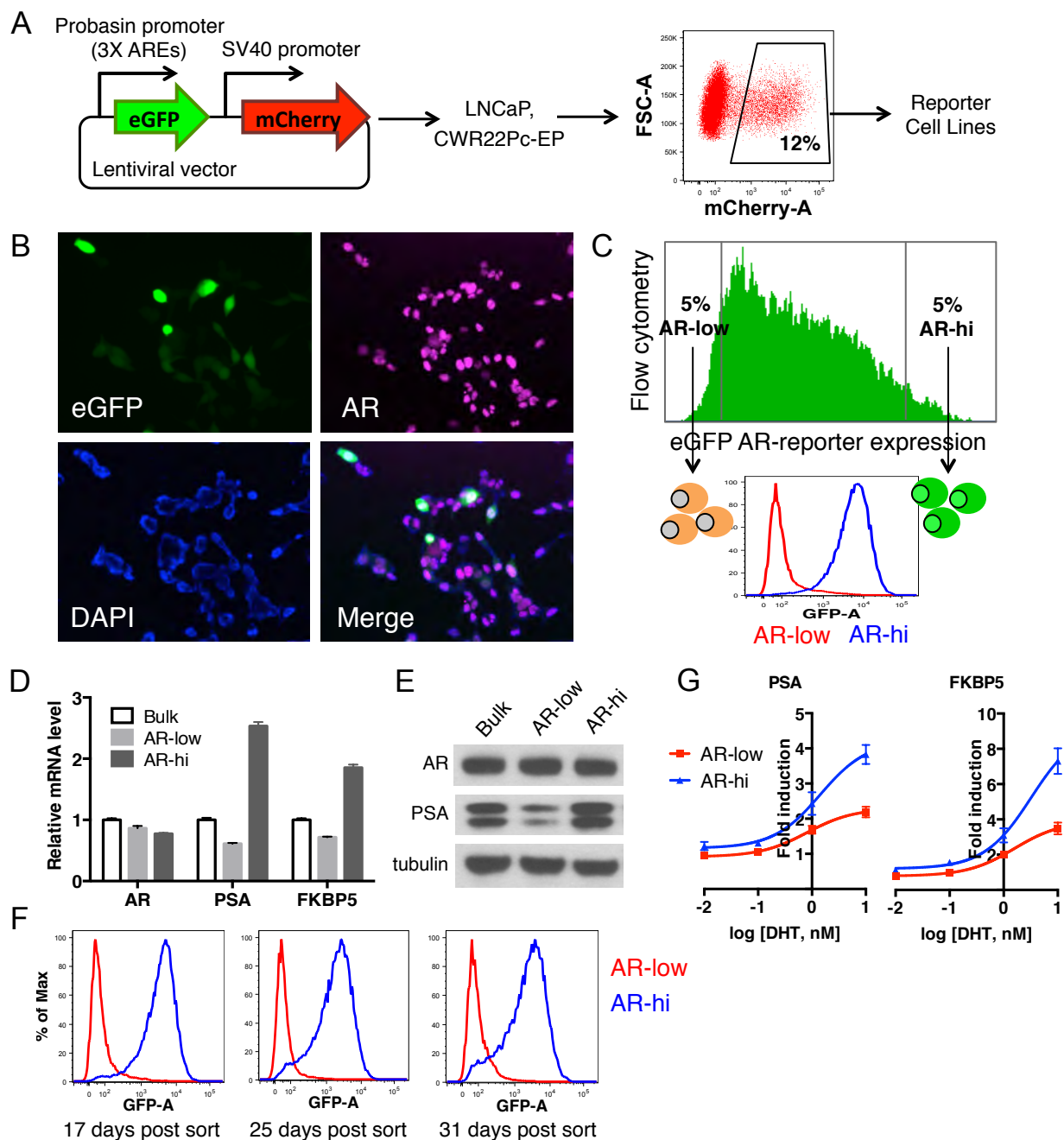
Figure 1. Characterization of LNCaP prostate cancer cells with low vs. high AR activity. (A) The reporter cell lines were generated by infecting the cells with the lentivirus containing eGFP AR reporter construct (details can be found in materials and methods). Cells stably integrated with the construct were sorted out by mCherry flow cytometry. (B) The variable AR reporter activity (green) in LNCaP cells. Cells were also stained with AR (magenta) and DAPI (blue). (C) LNCaP cells with low (AR-low) and high (AR-hi) AR activity were sorted out using flow cytometry based on eGFP AR-reporter expression. (D-E) AR-hi cells have higher AR output while having same level of AR. (F) AR-low and AR-hi cells maintain their AR activity over time. (G) AR-hi cells showed enhanced up-regulation of AR target genes in response to DHT treatment.

1-2. Compare enzalutamide sensitivity between cells with low vs. high AR activities

Proposed completion date: 03.2016-08.2016 (6 months)

Actual completion date: 03.2016-08.2016 (6 months)

Percentage of completion: 100%

Results: Having demonstrated the heterogeneity in AR output in prostate cancer cell lines, we next tested if the isolated AR-low and –hi populations have different biological behaviors. Interestingly, the *in vitro* growth assay showed that AR-low cells grow faster (Figure 2A) than bulk and AR-hi populations. Furthermore, contrary to our prediction that AR-low cells will be less dependent on AR activity, Figures 2B and C show that AR-low cells are in fact more sensitive to enzalutamide than AR-hi cells. To evaluate the tumor growth *in vivo*, we generated xenografts from bulk, AR-low or –hi populations. Consistent with the *in vitro* results, the tumors derived from AR-low population showed enhanced growth (Figure 2D). When we examined eGFP AR reporter activity of each tumor, the tumors derived from AR-low and -hi cells maintained their relative low and high AR activities compared to each other (Figure 2E). To test the enzalutamide sensitivity, we started the drug treatment when the xenografts reached ~200mm^3 and analyzed the volume change of each tumor during the 4 weeks of treatment. The result shows that although tumors derived from AR-low cells had bigger regression, enzalutamide significantly suppressed the tumor growth of all three groups (Figure 2F).
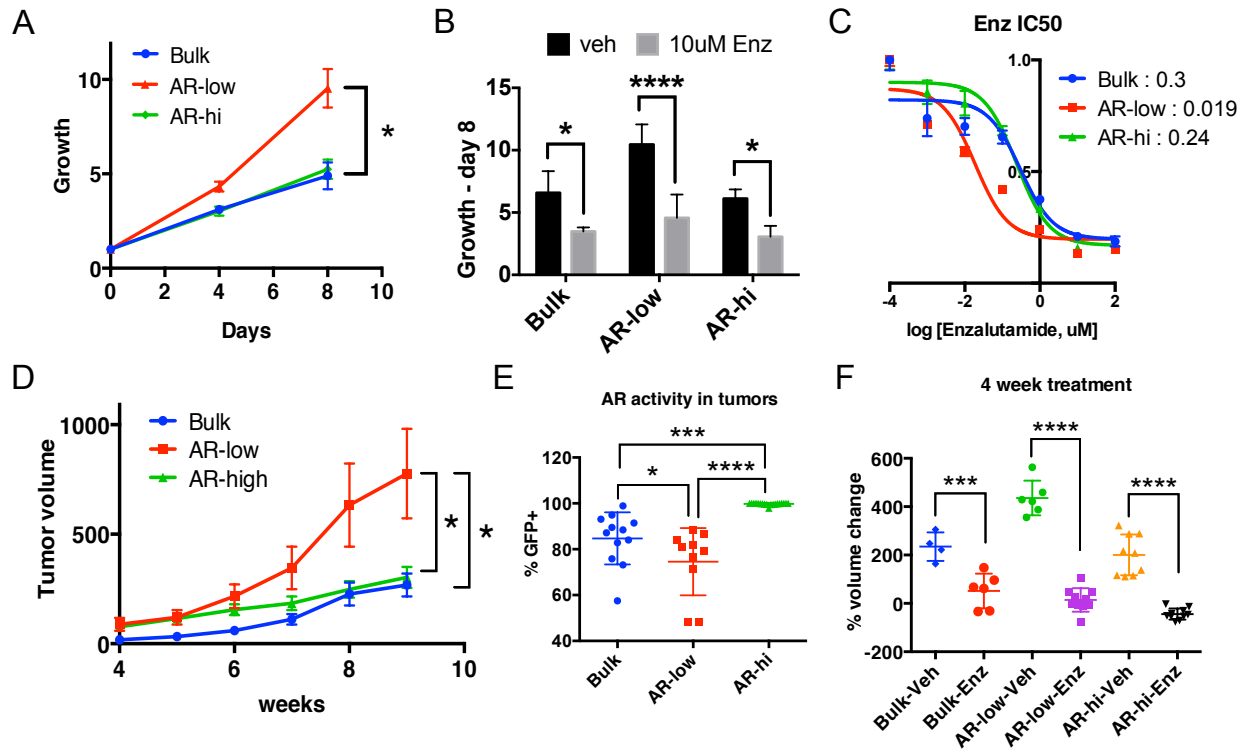
Figure 2. LNCaP prostate cancer cells with low AR activity grow faster and are more sensitive to enzalutamise. (A-B) AR-low cells grow faster than AR-hi cells (A) and more sensitive to enzalutamide (Enz) (B). (C) AR-low cells have lower IC50 value of enzalutamide compared to AR-hi cells. (D) Tumors derived from AR-low cells grow faster than tumors derived from other populations. (E) At the end the experiment described in (D), the percentage of the cells expressing GFP AR reporter were analyzed in individual tumor. (F) Enzalutamide treatment suppressed growth of the tumors derived from all three populations, although tumors derived from AR-low cells showed bigger regression. When the tumors reached ~200mm[3,] the mice were castrated and treated with vehicle (Veh) or enzalutamide (Enz). The volume change of each tumor was analyzed during the 4 weeks of the treatment.

Specific Aim 2: Identify the origin of enzalutamide resistant tumors with low/absent AR

Determine if the resistant tumors with low/absent AR are derived from pre-existing AR-low cells or transdifferentiation/reprogramming of AR-hi cells

Proposed completion date: 03.2016-10.2016 (8 months)

Actual completion date: 03.2016-08.2016 (6 months)

Percentage of completion: 100%

Results: Having showed that AR-low cells grow faster and more enzalutamide-sensitive, we next determined time to acquire enzalutamide resistance. We previously showed that a xenograft model derived from LNCaP cells over-expressing AR (LNCaP/AR) is a clinically relevant system to study the mechanisms of enzalutamide resistance (17, 18). We therefore used this model to compare enzalutamide resistance between AR-low and –hi populations. Consistent with the parental LNCaP cells, AR-low and –hi cells derived from LNCaP/AR cells showed different AR output but similar level of AR (data not shown). When the cells were injected into castrated mice and treated with enzalutamide immediately after injection, tumors derived from AR-hi cells became resistant to this treatment faster than other groups (Figure 3A). Interestingly, the AR activities in tumors derived from AR-hi cells were still higher than the ones from AR-low cells after the long-term enzalutamide treatment (Figure 3B).

These results suggest that 1) opposite to our original hypothesis, AR-hi cells have higher capacity to acquire enzalutamide resistance (Figure 3A), and 2) also contrary to our expectation that AR-hi cells might transdifferentiate/be reprogrammed to AR-low status during enzalutamide treatment, AR-hi tumors maintained their higher AR activity compared to AR-low tumors during the treatment (Figure 3B).
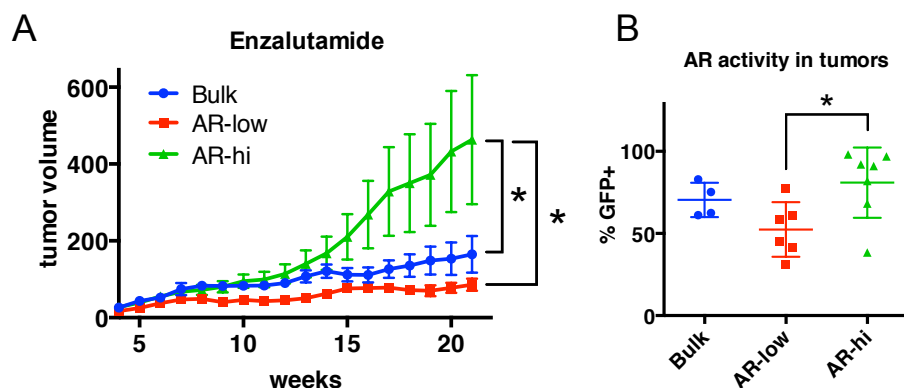

Figure 3. LNCaP/AR prostate cancer cells with high AR activity have capacity to acquire resistance to enzalutamide faster. (A) Tumors derived from LNCaP/AR AR-hi cells become resistant to enzalutamide faster than other populations. The sorted AR-low and –hi cells were injected into castrated mice and treated with enzalutamide immediately after injection. (B) At the end the experiment described in (G), the percentage of the cells expressing GFP AR reporter were analyzed in individual tumor.

Specific Aim 3: Uncover the molecular mechanisms of enzalutamide resistant tumors with low/absent AR

3-1. Analyze gene expression profile of AR-low and AR-hi cells using RNA-sequencing

Proposed completion date: 09.2016-10.2016 (2 months)

Actual completion date: 09.2016-10.2016 (2 months)

Percentage of completion: 100%

Results: Having demonstrated that AR-low and –hi cells have different biological characteristics, next we determined the molecular features of the two populations. The RNA-sequencing data showed that 69 genes were upregulated in AR-low cells and 191 genes were upregulated in AR-hi cells (Fold change $\geq$ 1.5, p < 0.05). Further gene set enrichment analysis (GSEA) revealed that the gene set upregulated by androgen was most enriched in AR-hi cells and the gene sets correlated with proliferation and cell cycle were most enriched in AR-low cells (Figure 4A), consistent with the enhanced growth of this population (Figure 2A,D). We also found that genes upregulated in human prostate luminal and basal cells were enriched in AR-hi and –low cells, respectively (Figure 4B), suggesting that AR might activate different set of genes in different subset of prostate cancer cells.
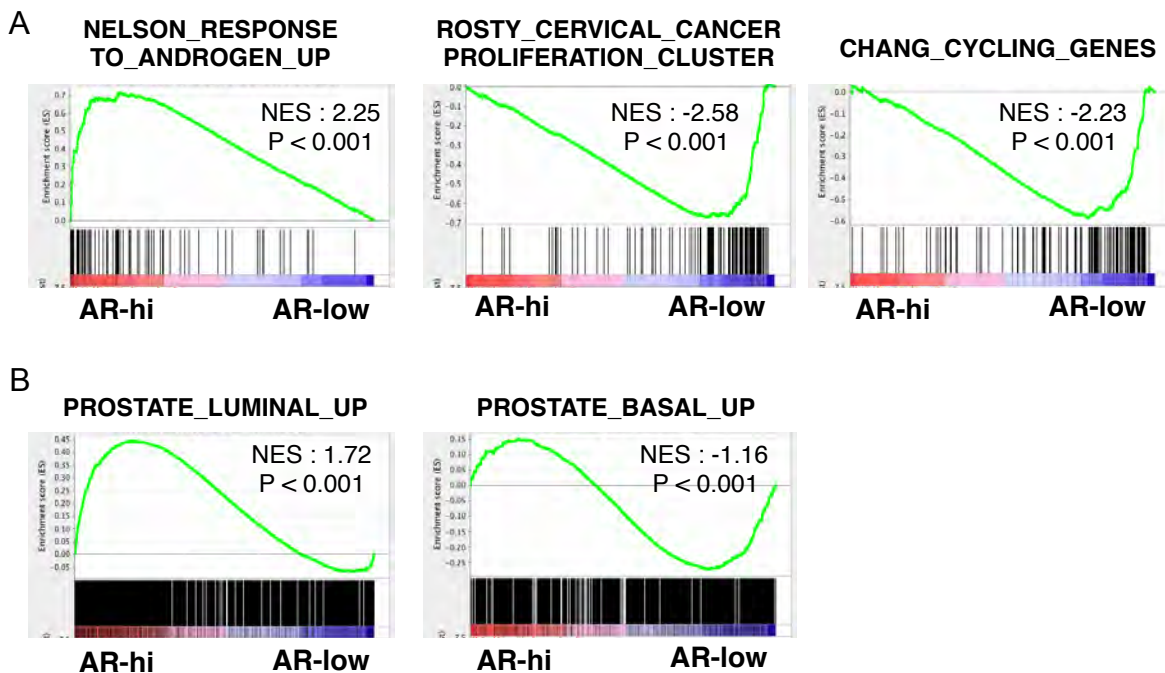

Figure 4. The gene expression profile of LNCaP cells with low vs. high AR activity. (A) Gene set enrichment analysis (GSEA) shows that the gene set upregulated by androgen is most enriched in AR-hi cells (left graph) and gene sets related to proliferation an cell cycle are most enriched in AR-low cells (two right graphs). (B) GSEA also shows that genes upregulated in human prostate luminal and basal cells are enriched in AR-hi and –low cells, respectively.

3-2. Identify factors important for survival of AR-low cells using RNA interference and/or pharmacological inhibition studies

Proposed completion date: 11.2016-06.2017 (8 months)

Actual completion date: 11.2016-01.2017 (3 months)

Percentage of completion: 100%

Results: We initially hypothesized that AR-low cells might be more resistant to enzalutamide treatment and proposed to identify genes/pathways important for the growth of AR-low cells. However, our data shows the opposite results that AR-low cells are in fact more sensitive to enzalutamide (Figure 2B and C), and the tumors derived from AR-hi cells becomes resistant to enzalutamide faster than AR-low cells (Figure 3A). Therefore, in this subtask, we further investigated the molecular characteristics of AR-hi cells, instead of AR-low cells.

To determine the mechanism for the higher AR output in AR-hi vs. –low cells, we focused on the fact that prostate luminal cell gene signature was enriched in AR-hi cells, since similar to AR-low and –hi cells, AR is expressed in both prostate basal and luminal cells but the AR signaling is much higher in luminal cells. Therefore, we identified 25 genes upregulated in both AR-hi and luminal cells (Appendix Table 1), and knocked down each gene in AR-hi cells (Figure 5A). We also knocked down 8 additional transcription factor/cofactors upregulated in AR-hi cells (Appendix Table 1 and Figure 5A) we identified using PANTHER classification system (19). The results showed that the knockdown of GREB1, GHRHR or KLF8 inhibited AR activity in AR-hi cells (Figure 5B, knockdown level of each gene in Figure 5C). We also found that each of these genes is regulated by AR (Figure 5D), which explains their increased expression in AR-hi cells. In fact, KLF8 was previously shown to interact with AR to promote AR transcriptional activity (20). Antagonists of GHRH were shown to suppress prostate cancer cell growth through inhibition of ERK and AKT pathways (21) but the affect of GHRHR inhibition on AR activity has not been studied. GREB1 is a known target of both ER and AR (22, 23), and was shown to promote ER transcriptional activity in breast cancer (24).

Among the three, GREB1 emerged as the most compelling candidate for further investigation. Besides its reported function as an ER cofactor, when we compared the RNA level of those three genes between TCGA primary prostate tumors with lowest and highest AR score (Figure 4E, 5% of 333 cases: 17 cases each), GREB1 was the only gene upregulated in tumors with high AR score consistent with the *in vitro* data (Figure 5F). Based on the consistency between *in vitro* and clinical data, and its reported function as an ER cofactor, we selected GREB1 for more detailed characterization.
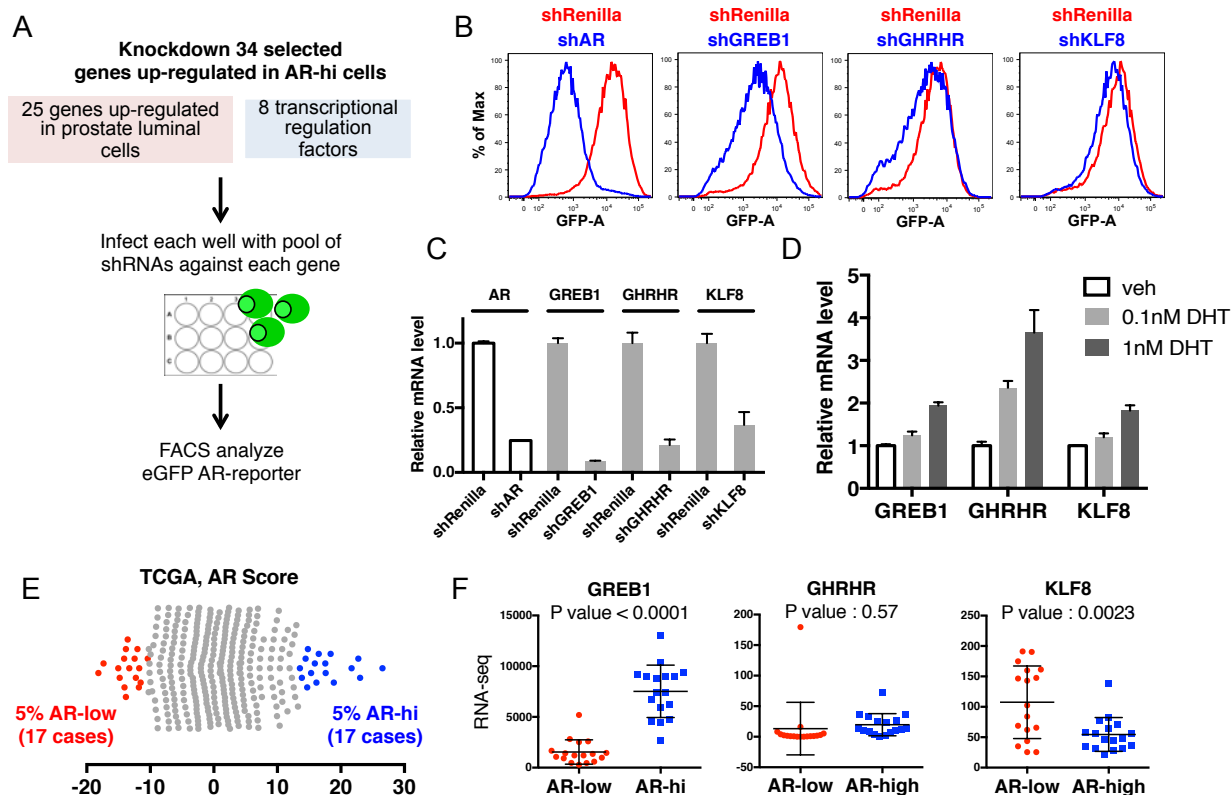
Figure 5. Knockdown of the three AR regulated genes, GREB1, GHRHR and KLF8, inhibited AR activity in cells with high AR activity. (A) The schematic of knockdown study with 34 selected genes up-regulated in AR-hi cells to find factors regulate AR-transcriptional activity in AR-hi cells. (B) The flow cytometry results show that the knockdown of GREB1, GHRHR and KLF8 inhibited AR reporter activity. AR shRNA was used as a control. (C) The knockdown level of GREB1, GHRHR and KLF8 in (B). (D) The transcription of GREB1, GHRHR and KLF8 is regulated by androgen. (E) Graph showing AR score of each TCGA primary prostate tumor. Red and blue points are tumors with lowest (AR-low) and highest (AR-hi) AR score, respectively (5% of 333 cases: 17 cases each). (F) The RNA levels of GREB1, GHRHR and KLF8 are compared between AR-low vs. AR-hi TCGA cases.

3-3. Identify the molecular regulators of transdifferentiation/reprogramming of AR-hi cells to enzalutamide resistant tumors with low/absent AR

Proposed completion date: 11.2016-09.2017 (10 months)

Actual completion date: 11.2016-09.2017 (10 months)

Percentage of completion: 100%

Results: We initially hypothesized that AR-hi cells might be reprogrammed to AR-low status under enzalutamide treatment to become resistant to AR inhibition. However, our data shows that the enzalutamide resistant AR-hi tumors maintained their higher AR activity compared to AR-low tumors (Figure 3B). Therefore, in the previous subtask, we identified factors responsible for higher AR activity in AR-hi cells using knockdown study in combination with RNA-sequencing (Figure 5). As a result, we found GREB1 as a potential new AR cofactor and further investigated the molecular function of GREB1 in prostate cancer in this subtask.

GREB1 was first reported as an estrogen-regulated gene in breast cancer (22), but its exact function or structure is not known. A recent mass spectrometry study showed that GREB1 is the most estrogen-enriched ER-interacting protein, presumably through its LxxLL motif, promoting ER activity by enhancing ER interaction with other cofactors such as p300/CBP complex (24). Therefore, we determined the effect of GREB1 on AR transcriptional activity in prostate cancer. When we transfected increasing amount of GREB1 plasmid in LNCaP cells, we observed dose response increase of luciferase AR reporter activity (Figure 6A), suggesting that GREB1 also promotes AR activity. We next tested if GREB1 is responsible for the higher AR output in AR-hi cells. When we knocked down GREB1 in both AR-low and-hi cells, GREB1 depletion suppressed AR target gene expression in both populations (Figure 6B,C), suggesting that the effect of GREB1 knockdown is not limited to AR-hi cells. However, GREB1 knockdown in AR-hi cells inhibited the enhanced DHT-induced AR target gene upregulation (Figure 6D) observed in this population (Figure 1G), suggesting that GREB1 is accountable for the higher AR activity in AR-hi cells. Similarly, when we stably integrated AR-low cells with control or GREB1 expression vector (HA-GREB1) (Figure 6E), GREB1 overexpression enhanced DHT-induced AR target gene upregulation in AR-low cells (Figure 6F) and also increased protein levels of AR target genes without affecting AR expression (Figure 6G). These results suggest that GREB1 is an AR cofactor upregulated in prostate cancer cells with high AR activity.

To further investigate the role of GREB1 on AR transcriptional activity, AR-hi cells with control or GREB1 shRNA were subjected to RNA-sequencing. As expected, GSEA revealed that the gene set upregulated by androgen was most enriched in control cells compared to GREB1 depleted cells (Figure 6H), suggesting that the main function of GREB1 in prostate cancer is promoting AR signaling. We next compared the expression of 20 AR target genes used to analyze AR score (10), and observed robust downregulation of this gene set in GREB1 knockdown cells (Figure 6I). We then analyzed the number of genes upregulated by DHT in each group, which showed that 70.5% of DHT-induced genes in control cells were inhibited by GREB1 depletion (Figure 6J). The reduced AR signaling resulted in suppressed cell proliferation (Figure 6K), consistent with the previous report (23).
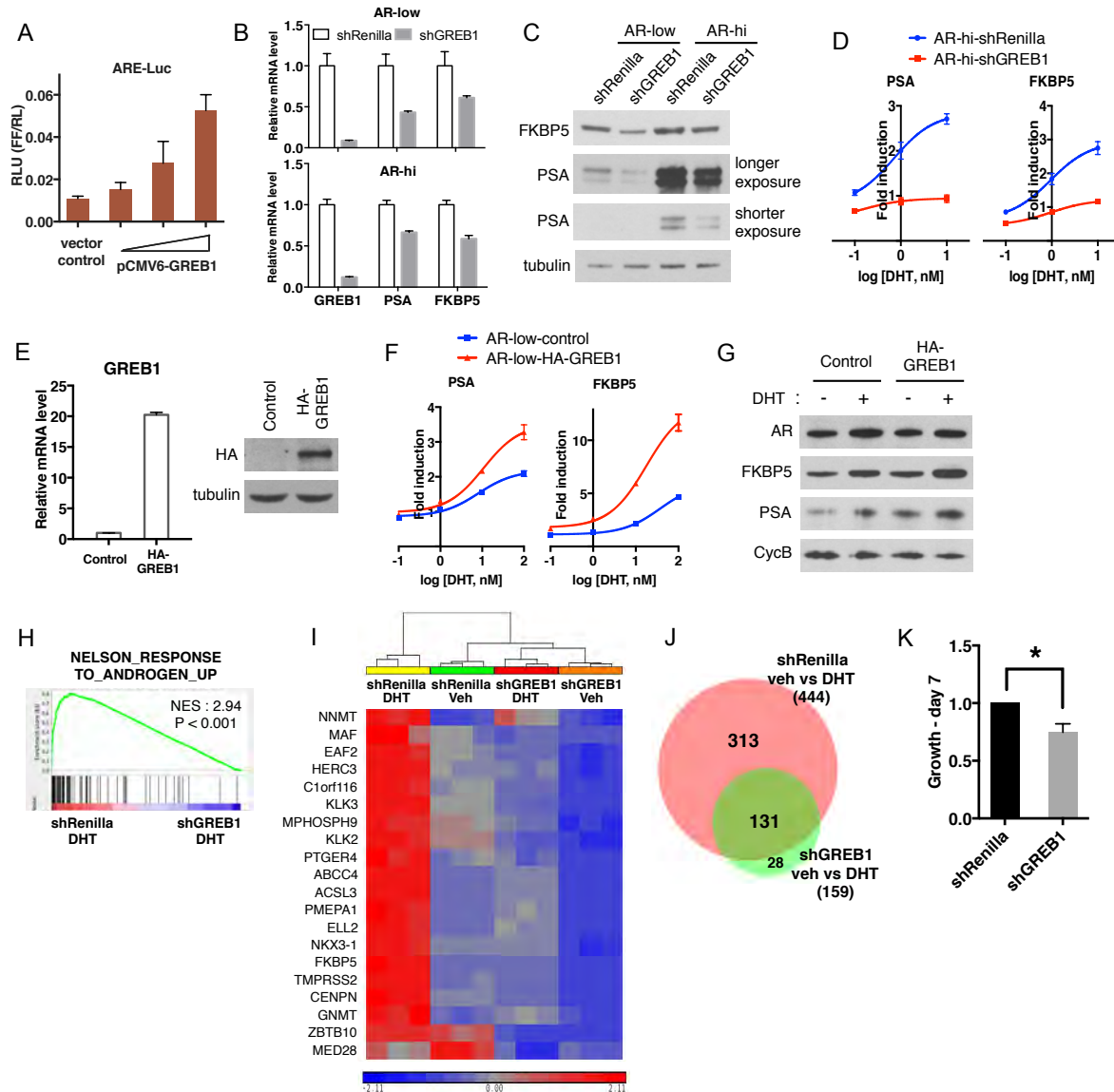
Figure 6. GREB1 amplifies AR transcriptional activity in LNCaP cells. (A) Transfection of GREB1 plasmid in LNCaP cells shows dose response increase of luciferase AR reporter activity. (B-C) Knockdown of GREB1 inhibited AR target gene expression in both AR-low and -hi cells. (D) Knockdown of GREB1 suppressed the enhanced AR transcriptional activity in AR-hi cells. (E) GREB1 overexpression in AR-low cells with stable integration of GREB1 vector containing HA-tag. (F) AR-low cells with GREB1 overexpression show higher induction of AR target genes in response to DHT treatment. (G) GREB1 overexpression in AR-low cells increases protein levels of AR target genes without affecting AR level. (H) Gene set enrichment analysis (GSEA) shows that the gene set up-regulated by androgen is most enriched in control cells compared to GREB1 depleted AR-hi cells. (I) The heatmap generated from the gene expression data shows that the expression of 20 AR target genes used to calculate AR score is suppressed by GREB1 knockdown in AR-hi cells. (J) The venn diagram showing that 70.5% of DHT-induced genes in control AR-hi cells was inhibited by GREB1 knockdown. (K) Knockdown of GREB1 suppressed growth of AR-hi cells.

We next investigated how GREB1 regulates AR transcriptional activity. In breast cancer, GREB1 was recruited to ER binding sites and mediated ER interaction with p300/CBP complex (24). To determine if GREB1 interacts similarly with AR, we utilized LNCaP cells overexpress HA-tagged GREB1 and found AR interaction with GREB1 (Figure 7A) and GREB1 binding to PSA and FKBP5 enhancer regions (Figure 7B). Furthermore, AR-hi cells showed increased p300 binding (Figure 7C), which was dependent on GREB1 (Figure 7D). These results suggest that similar to ER, GREB1 also promotes AR transcriptional activity by recruiting p300 to AR.

However, inconsistent to ER, we found that GREB1 knockdown inhibited AR binding to its binding site (Figure 6E). To further examine the effect of GREB1 on AR binding to DNA, we performed AR ChIP-sequencing in AR-low and -hi cells with control or GREB1 knockdown, and observed reduced AR peaks across AR binding sites in both cell types (Figure 6F,G).
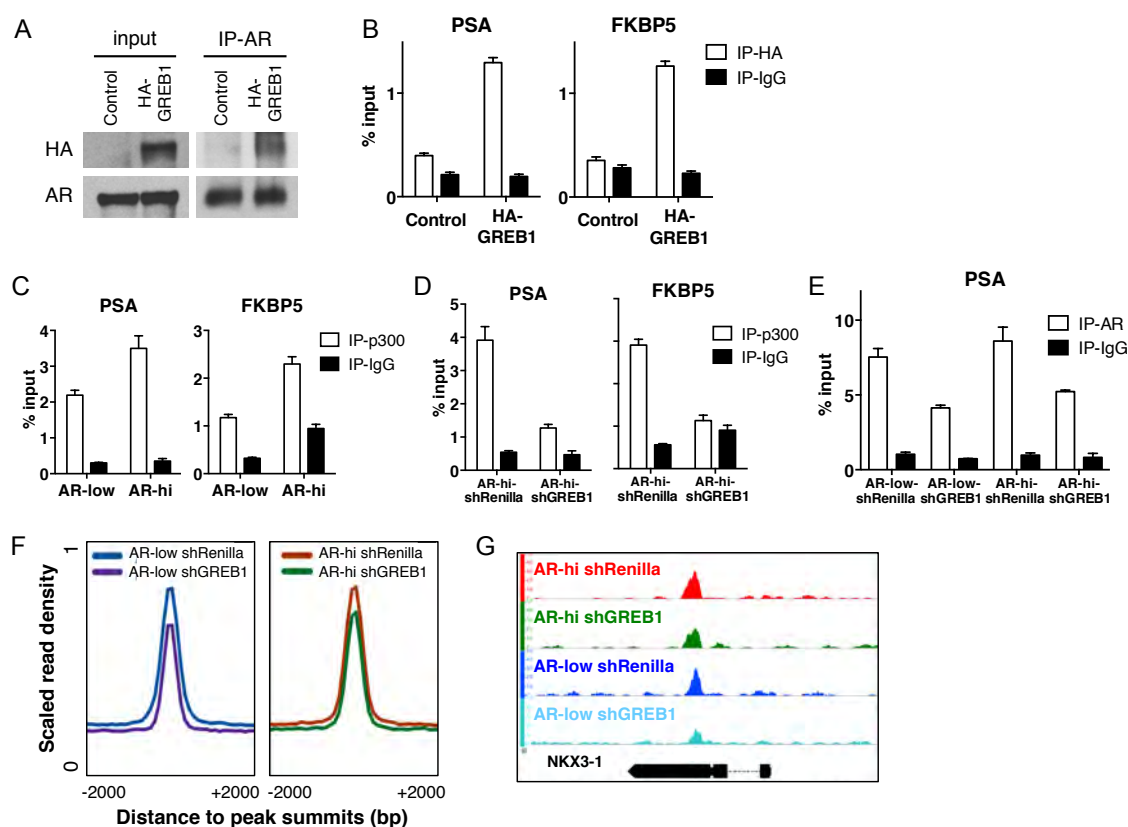

Figure 7. Knockdown of GREB1 in LNCaP cells inhibits p300 recruitment to AR target genes and AR binding to chromatin. (A) Coimmunoprecipitation study using nuclear extract shows interaction between AR and GREB1 (HA). (B) ChIP against HA-tag shows GREB1 binging on PSA and FKBP5 enhancer regions. (C-D) AR-hi cells have increased p300 binding on PSA and FKBP5 enhancer regions in a GREB1 dependent manner. (E) GREB1 knockdown decreases AR binding to PSA enhancer. (F) ChIP-sequencing summary plot shows that AR enrichment across the AR binding sites are reduced by GREB1 knockdown in both AR-low and –hi cells. (G) Example AR peaks on NKX3-1 gene.

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Many studies have shown that AR amplification in prostate tumor is associated with poor clinical response to AR targeted therapy (3, 4, 25). Therefore, having demonstrated that GREB1 amplifies AR transcriptional activity, we next examined GREB1 alteration in patient data set. In SU2C metastatic CRPC cohort (4), we found that AR and GREB1 alterations are mutually exclusive in a significant level (Figure 8A). Given that GREB1 alterations are mostly mRNA upregulation and few mutations (Figure 8A), we asked if GREB1 alteration alone without AR alteration can restore AR signaling during AR targeted therapy and promote resistant to the treatment. To test this, we compared AR score between SU2C cases with AR or GREB1 alteration alone and cases with no alteration of both genes and found that the AR scores in GREB1 alone-altered cases are higher than unaltered cases and similar to the ones in AR alone-altered cases (Figure 8B). To further investigate the potential role of GREB1 in treatment resistance, we tested the effect of GREB1 overexpression on enzalutamide sensitivity. The growth assay shows that GREB1 overexpression increased the IC50 value of enzalutamide in both LNCaP and CWR22Pc-EP cells (Figure 8C). Importantly, GREB1 level was increased in the SU2C cases received next-generation AR therapies, abiraterone and/or enzalutamide treatment (Figure 8D), suggesting that GREB1 might have a functional role in disease progression.
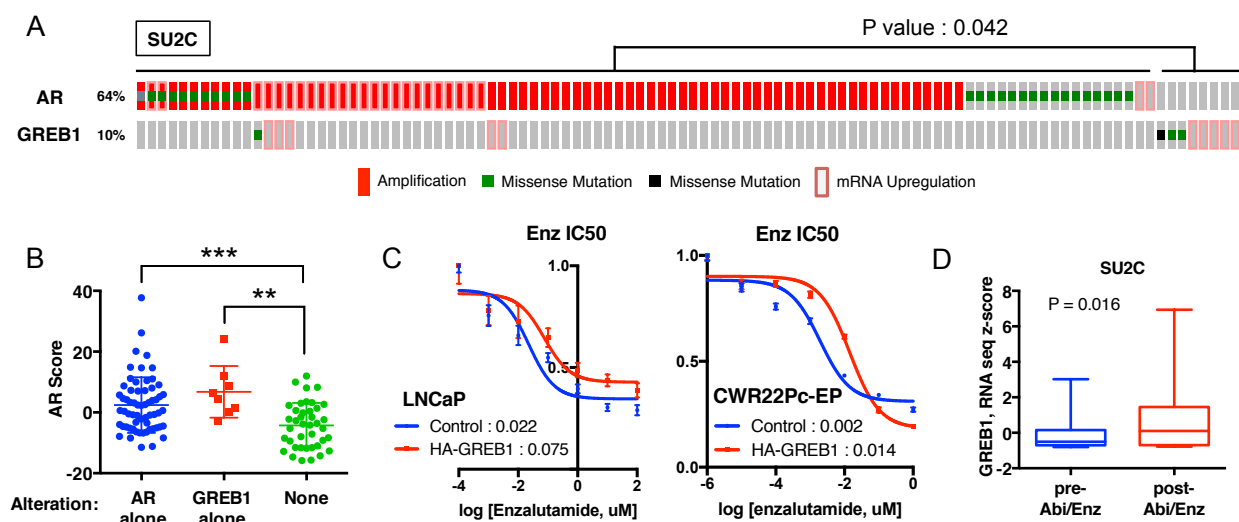

Figure 8. GREB1 amplified AR transcriptional activity in prostate tumors and modifies enzalutamide sensitivity. (A) cBioPortal OncoPrint summary shows that AR and GREB1 alterations are mutually exclusive in SU2C metastatic CRPC cohort. (B) AR scores in GREB1 alone-altered SU2C tumors are higher than unaltered tumors and similar to the ones in AR alone-altered tumors. (C) GREB1 overexpression increased IC50 value of enzalutamide (Enz) in both LNCaP and CWR22Pc-EP AR-low cells. (D) The SU2C cases received next-generation AR therapies, abiraterone (Abi) and/or enzalutamide (Enz), have increased level of GREB1.

Opportunities for training and professional development:

The training goal for my post-doctoral fellowship is to strengthen my scientific independence by improving critical research thinking and experimental techniques. To do so, during the two years of training period, I attended internal and external meetings/conferences. In addition to the weekly lab meetings, I attended MSKCC seminar series featuring speakers in cancer biology and translational research. I also attended the annual American Association for Cancer Research (AACR) meeting where I had opportunity to share scientific ideas with eminent researchers across the world. During the two years of training period, I've learned technical skills related to the xenograft assay, RNA and ChIP-sequencing and also learned how to analyze the data I obtained from these studies. Also, by completing the small-scale shRNA screen and following up one of the hits, GREB1, I've learned how to validate the candidate molecular factors that might have clinical significance in disease progression.

Results disseminated to communities of interest:

Nothing to report

Plan for the next reporting period:

Nothing to report

Impact

Impact on the development of the principal disciplines of the project:

Prostate cancer is the most common form of cancer in men. Given the key role of androgen receptor (AR) signaling in disease progression, the current approach to treat prostate cancer is AR-targeted therapy. While this initially results in tumor regression, aggressive disease typically recurs, making the treatment of what is now called castration-resistant prostate cancer (CRPC) the major challenge in the field (26). Charles Sawyers' laboratory developed a second-generation AR inhibitor, enzalutamide (6), that has increased both patient survival and quality of life (27). However, resistance remains as a major problem with complex molecular mechanisms (17, 28), reflecting the heterogeneity of the disease. Increasing number of studies have shown the molecular and genetic diversity among prostate cancers (4, 11) including a wide range of AR activity (10). Given that AR is the central therapeutic target in this disease, this observation lead to an important question if tumors with different AR activity have differential response to AR-targeted therapy. Indeed, one of the resistance mechanisms to enzalutamide is that a small subset of resistant tumors shows low or absent AR expression (29), which can explain the nonresponsiveness of tumors to AR inhibition. Given this, we hypothesized that the prostate cancer with low AR activity will be more sensitive to enzalutamide treatment. Our data shows that the results are in fact the opposite, that the tumors with higher AR activity are more resistant to enzalutamide treatment. We also identified GREB1 as an AR cofactor upregulated in prostate cancer with higher AR activity and potentially promote resistance to enzalutamide treatment.

The results of this work will provide valuable information on developing therapeutic strategies for prostate cancer patients. Our data suggests that in primary prostate cancer where there are rare events of AR amplification, the level of AR activity might be able to distinguish aggressive disease. Given that GREB1 was upregulated in tumors with higher AR activity, GREB1 can be used as a marker to screen prostate cancer with high AR activity (high GREB1) that might not be benefitted from enzalutamide treatment. Furthermore, our data showed that inhibition of GREB1 function in these tumors with high AR activity suppressed the cell growth, indicating the therapeutic potential of targeting GREB1. Therefore, further understanding of the function of GREB1 in prostate cancer will provide novel insights into the development of effective therapeutic approaches, which will ultimately decrease suffering and improve survival of prostate cancer patients.

Impact on the other disciplines:

Nothing to report

Impact on technology transfer:

Nothing to report

Impact on society beyond science and technology:

Nothing to report

Changes/Problems

Changes in approach and reasons for change:

In our initial proposal, we hypothesized that the AR-low prostate cancer cells might be more resistant to enzalutamide treatment and proposed to further study this cell population. However, our data showed the opposite result that AR-low cells are in fact more sensitive to enzalutamide (Figure 2B,C), and tumors derived from AR-hi cells acquire resistance to enzalutamide faster than AR-low cells (Figure 3A). We also proposed to study the enzalutamide resistant AR-low tumors that are derived from AR-hi cells, based on the hypothesis that AR-hi cells might be reprogrammed to AR-low status under AR-targeted therapy to become resistant to this treatment. However, our data showed that the enzalutamide resistant AR-high tumors maintained their higher AR activity compared to AR-low tumors (Figure 3B). Therefore, we changed our approach and attempted to identify factors responsible for higher AR activity in AR-hi cells using knockdown study in combination with RNA-sequencing (Figure 5). As a result, we found GREB1 as a potential new AR cofactor and further investigated the molecular function of GREB1 in prostate cancer (Figure 6-8).

Actual or anticipated problems or delays and actions or plans to resolve them:

Nothing to report

Changes that had a significant impact on expenditures:

Nothing to report

Changes in use or care of human subjects, vertebrate animals, biohazards, and select agents:

Nothing to report

Product

Publications, conference papers, and presentations:

Nothing to report

Website(s) or other Internet site(s):

Nothing to report

Technologies or techniques:

Nothing to report

Inventions, patent applications, and/or licenses:

Nothing to report

Other Products:

Nothing to report

Participants & other collaborating organizations

Individuals worked on the project:

Name: Eugine Lee
Project Role: PI
Nearest person month worked: 24
Contribution to Project: Dr. Lee has designed and performed all of the experiments during the periods.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report

Special reporting requirements

None.

Appendices

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Table 1. List of genes targeted in small-scale shRNA screen described in specific aim 3-2.

25 genes upregulated in both prostate luminal cells and LNCaP AR-hi cells	8 transcription factors/cofactors upregulated in LNCaP AR-hi cells
GHRHR	ID4
KLHL41	ELL2
TXNIP	ZFPM2
AGR2	SP100
RBP4	KLF8
MYBPC1	SOX6
CRYAB	LHX9
DPP4	SP9
EPDR1	
PDE11A	
FAM105A	
STS	
LIPH	
PGC	
AZGP1	
ACPP	
SLC9A9	
CASP4	
MICAL1	
GREB1	
AFF3	
ARRDC4	
HIST1H2AI	
PPAP2A	
STEAP4	